

Supplementation of Zinc and Vitamin E on Apparent Digestibility of Nutrient, Carcass Traits, and Mineral Availability in Broiler Chickens

T. F. Rayani^a, R. Mutia^{b*}, & Sumiati^b

^aStudy Program of Nutrition and Feed Science, Faculty of Animal Science, Graduate School, Bogor Agricultural University

^bDepartment of Nutrition and Feed Science, Faculty of Animal Science, Bogor Agricultural University

Jalan Agatis, Kampus IPB Darmaga Bogor 16680, Indonesia

(Received 16-08-2016; Reviewed 10-10-2016; Accepted 03-02-2017)

ABSTRACT

The purpose of this study was to determine the effect of zinc (Zn) and vitamin E supplementations on apparent nutrient digestibility, carcass traits, and mineral availability of broiler in tropical environment. The experiment was arranged in a 3 x 3 factorial design with 4 replications in each treatment (10 birds each). The dietary treatments were the combination of doses of zinc (A1: 0 ppm, A2: 40 ppm, A3: 80 ppm) and vitamin E (B1: 0 ppm, B2: 125 ppm, B3: 250 ppm) supplementations. The variables observed were apparent nutrient digestibilities (apparent crude protein digestibility and apparent ether extract digestibility), carcass traits (final weight, the percentage of carcass weight, breast weight, thigh weight, wings weight, and back weight), meat bone ratio and mineral availability in the tibia (calcium [Ca], phosphorus [P], and zinc [Zn]). The results showed that apparent nutrient digestibility, carcass traits (the percentages of carcass, breast, thighs, wings, and back), meat bone ratio were not significantly influenced by the supplementation of zinc or vitamin E. The supplementation of 80 ppm of Zn and 0 ppm of vitamin E significantly ($P < 0.05$) increased final weight of broiler. Supplementation of zinc alone and vitamin E alone in the diet significantly ($P < 0.05$) increased calcium content of the tibia. In conclusion, supplementation of 80 ppm of Zn increased calcium content compared to control, but did not affect final weight and ether-extract digestibility. Supplementation of vitamin E at a dose of 125 ppm could increase calcium content of the tibia compared to control.

Keywords: broiler, vitamin E, zinc

ABSTRAK

Tujuan penelitian ini adalah untuk mengevaluasi pengaruh suplementasi mineral zink (Zn) dan vitamin E pada pencernaan semu nutrisi, kualitas karkas, dan kadar mineral pada ayam broiler yang dipelihara di lingkungan tropis. Penelitian menggunakan rancangan faktorial 3 x 3 dengan 4 ulangan di setiap perlakuan (10 ekor per ulangan). Perlakuan pada penelitian ini merupakan kombinasi suplementasi mineral zink (A1: 0 ppm, A2: 40 ppm, dan A3: 80 ppm) dan vitamin E (B1: 0 ppm, B2: 125 ppm, dan B3: 250 ppm). Variabel yang diukur adalah pencernaan nutrisi semu (pencernaan protein kasar semu dan pencernaan lemak kasar semu), kualitas karkas (berat hidup akhir, persentase karkas, dada, paha, sayap, dan punggungan), rasio daging dan tulang dada, dan kadar mineral pada tulang tibia (kalsium, fosfor, dan zink). Hasil penelitian menunjukkan bahwa pencernaan nutrisi semu, kualitas karkas (persentase karkas, dada, paha, sayap, dan punggungan), rasio daging dan tulang dada tidak dipengaruhi secara nyata oleh penambahan mineral zink dan vitamin E pada ransum. Penambahan kombinasi Zn dengan dosis 80 ppm dan vitamin E dengan dosis 0 ppm berpengaruh nyata ($P < 0.05$) meningkatkan bobot badan akhir ayam broiler. Penambahan mineral zink atau vitamin E secara tunggal dalam ransum berpengaruh nyata ($P < 0.05$) meningkatkan kadar kalsium dalam tulang tibia, tetapi tidak ada pengaruh penambahan kombinasi keduanya. Kesimpulan dari penelitian ini adalah penambahan mineral zink sebesar 80 ppm meningkatkan kadar kalsium dalam tulang tibia, tetapi tidak berpengaruh terhadap bobot badan akhir dan pencernaan lemak kasar. Pemberian vitamin E sebanyak 125 ppm mampu meningkatkan kadar kalsium dalam tulang tibia ayam broiler dibanding kontrol.

Kata kunci: ayam broiler, mineral zink, vitamin E

*Corresponding author:

E-mail: rmutia.1@gmail.com

INTRODUCTION

Ambient temperature is one of important factors that must be considered in the production of broiler chickens. Indonesia as a tropical country has a high ambient temperature that can cause heat stress in broiler. When the heat received by the body from the environment is higher than the heat loss from the body, then the body temperature will increase and cause heat stress (Sudarman *et al.*, 2011). Heat stress affects physiological and hormonal changes and causes a decrease in feed intake. Heat stress also increases mineral excretion, and decreases concentrations of vitamin and minerals in the serum and liver (Sahin & Krucuk, 2003). High environmental temperature is detrimental to health and decreases immunity, and nutrient availability, which will subsequently result in low growth rates (Sahin *et al.*, 2008; Lara & Rostagno, 2013). The initial responses of birds suffering from heat stress are the decreased feed intake and high consumption of drinking water. High ambient temperature also decreases nutrient digestibility values. Heat stress is known to damage the barriers of digestive tract, lower blood flow by 50% in the proventriculus, gizzard, and pancreas, and by 70% in the upper duodenum and jejunum due to heat stress (Gu *et al.*, 2012). Heat stress significantly decreases the ash, Ca, and P content of the tibia bone and leads to the decreased bone strength (Sahin *et al.*, 2006; Hosseini-Vashan *et al.*, 2016). This factor can cause financial losses due to the decreased body weight gain, high feed conversion ratio, and mortality. Some efforts have been done to overcome those problems by adding feed additive such as antioxidant (Sahin *et al.*, 2008; Selim *et al.*, 2013. Habibian *et al.*, 2014; Rusli *et al.*, 2015). Recently, vitamin and mineral are used as antioxidants.

Vitamin E is the major antioxidant component in biological system which plays an important role in metabolism process, protecting cellular structure, and maintaining membrane stability from free radical. Vitamin E is one of the vitamins that plays an important role in the integrity of circulatory, muscular, reproductive, nervous, and immune systems of animals (Habibian *et al.*, 2014). Supplementation of vitamin E in broiler feed results in positive effects on growth performance during heat stress. Vitamin E supplementation was demonstrated to improve the quality of meat products, carcass, and cut yield, increase the activation of the immune system, and reduce the peroxidation of membrane lipids (Hashizawa *et al.*, 2013).

Zinc (Zn) has a major role as a component of many of metalloenzymes such as carbonic anhydrase, carboxypeptidases, and DNA polymerases (Liu *et al.*, 2011; Liu *et al.*, 2015; Salim *et al.*, 2008; Salim *et al.*, 2012). Rossi *et al.* (2007) reported that organic Zn (45 mg/kg, with addition of 60 mg ZnSO₄ in the diets) minimized skin tearing and improved carcass appearance of broiler. Triona *et al.* (2007) concluded in their study that organic Zn (Zn-glycine) increased the proportion of breast and leg muscles, dressing percentage, and fat content in the breast and leg muscles of broiler. Deficiency of Zn can cause a reduction in weight gain, skeletal malformations, poor bone mineralization, and immunological dysfunctions.

Accordingly, a study on the supplementation of the broiler chickens diets with zinc and vitamin E was carried out to determine their effects on apparent nutrient digestibility, carcass quality, and mineral availability.

MATERIALS AND METHODS

Design, Animal, and Diets

This research used 360 male day-old chicks (Lohman strain, Japfacomfeed, Indonesia). They were raised in the cages each with the size of 1.5 x 1.5 m. The experiment was arranged in a 3 x 3 factorial design with 4 replications (10 birds each) in each treatment. The dietary treatments were 3 levels of zinc (Zn) supplementation i.e., 0 ppm (A1), 40 ppm (A2), and 80 ppm (A3) and 3 levels of vitamin E (VE) supplementation i.e., 0 ppm (B1), 125 ppm (B2), and 250 ppm (B3). The diets and drinking water were provided *ad-libitum*. The feeding trial was conducted for 35 d.

A commercial ration (BR 511, CP 21%-23%, PT. Charoend Pokphand Indonesia) was used for starter periode (1-21 d) and the finisher diets (treatments diets) consisted of a mixture of maize (60.97%), soybean meal (26 %), fish meal (7%), coconut oil (4%), CaCO₃ (1.08%), NaCl (0.35%), premix (0.50%), and DL-Methionine (0.1%). Feed requirement of broiler was defined according to Leeson & Summers (2008) containing the metabolizable energy of 3100 kcal/kg and 20% of crude protein. The composition of the diets and nutrient contents used in this study are shown in Table 1. The birds were weighed and feed intake were determined on a weekly basis.

Variables Measured

Carcass traits. The variables measured in the measurement of the carcass traits were the live weight, the percentage of carcass weight, breast weight, thighs weights, wings weights, and back weight. The final live weights of the experimental chickens were the weights at the age of 35 days, a day before slaughtering, while the dressed carcass weights were obtained from chickens that were slaughtered without feather, blood, entrails, heads, and feet. Breast, thighs, wings, and back (dorsal) were weighed. The percentages of weight of breast, thighs, and back (dorsal) were calculated based on the body live weights of the birds.

Meat bone ratio of the breast. Meat bone ratio of the breast is a comparison of the abundance of meat in each unit of sternum. Meat bone ratio was obtained by separating meat and bone of the breast (g).

Calcium, zinc, and phosphorus retentions of tibia. The right and left tibia from each bird were pressure-cooked (100°C) for 20 min. The cooked tibia were cleaned manually, and the attached muscle and cartilage were removed, and the separated tibias were washed with water and oven dried at 60 °C for 24 h. Preparation of tibias for dry ashing mineral analysis followed the method of AOAC Official Methods of Analysis (2005). Preparation

of tibias for phosphorus analysis was carried out according to the method by Tausky HH and Short E (1953).

Apparent nutrient digestibility. The sampling of excreta was carried out based on the method of Farrell (1978) with a slight modification. The experimental broiler chickens were placed in metabolic cages and fasted for 24 hours. After fasting, the experimental broiler chickens were fed for 2 h and feed intakes were recorded. The excretas were collected over the next 24 h. Every four hours, the excretas were sprayed using a 0.01% of H₂SO₄ solution. The excretas produced were weighed and dried in an oven using a temperature of 60°C. The measurement of the apparent nutrient digestibility was done by measuring the content of dry matter, ether extract, and crude protein in the feed and excreta. Samples for dry matter, ether extract, and crude protein analyses were prepared in accordance with the procedure of AOAC (1995).

Data Analysis

Data were then statistically analyzed with a two-way Analysis of Variance (ANOVA) by using a software SPSS application (IBM®SPSS® version 21.0). Duncan Multiple Range Test (DMRT) analysis was used to determine the differences between means among the treatments (Steel & Torrie 1995). Differences were considered significant at $P < 0.05$.

RESULTS

Nutrient Digestibility

The effects of supplementations of Zn and vitamin E on nutrient digestibilities are shown in Table 2. There was no significant interaction effect of Zn and vitamin E supplementations on the digestibility of crude protein and ether-extract of the diet of broiler. Furthermore supplementation of Zn or vitamin E in the diet did not affect the nutrients digestibility.

Table 1. Nutrient contents of the experimental diets (calculated)

Nutrient contents ^a	Starter ^b	Finisher (%)								
		A1B1	A1B2	A1B3	A2B1	A2B2	A2B3	A3B1	A3B2	A3B3
Dry matter (%)	89.57	90.07	90.07	90.07	90.07	90.07	90.07	90.07	90.07	90.07
Ash (%)	5.97	4.90	4.90	4.90	4.90	4.90	4.90	4.90	4.90	4.90
Crude protein (%)	21.88	21.28	21.28	21.28	21.28	21.28	21.28	21.28	21.28	21.28
Crude fiber (%)	2.56	2.08	2.08	2.08	2.08	2.08	2.08	2.08	2.08	2.08
Ether extract (%)	6.31	5.35	5.35	5.35	5.35	5.35	5.35	5.35	5.35	5.35
Nitrogen free extract (%)	52.85	56.46	56.46	56.46	56.46	56.46	56.46	56.46	56.46	56.46
Ca (%)	1.83	1.54	1.54	1.54	1.54	1.54	1.54	1.54	1.54	1.54
P (%)	0.46	0.68	0.68	0.68	0.68	0.68	0.68	0.68	0.68	0.68
Gross Energy(kkal)	4348	4207	4207	4207	4207	4207	4207	4207	4207	4207
Vit E (ppm)	-	0	125	250	0	125	250	0	125	250
Zn (ppm) ^c	33.34	56.78	56.78	56.78	96.78	96.78	96.78	136.78	136.78	136.78

Note: A1B1= basal diet + 0 ppm Zn + 0 ppm VE; A1B2= basal diet + 0 ppm Zn + 125 ppm VE; A1B3= basal diet + 0 ppm Zn + 250 ppm VE; A2B1= basal diet + 40 ppm Zn + 0 ppm VE; A2B2= basal diet + 40 ppm Zn + 125 ppm VE; A2B3= basal diet + 40 ppm Zn + 250 ppm VE; A3B1= basal diet + 80 ppm Zn + 0 ppm VE; A3B2= basal diet + 80 ppm Zn + 125 ppm VE; A3B3= basal diet + 80 ppm Zn + 250 ppm VE. a) Premix content (in mg/kg premix): vit A 1200000 IU; vit D3 200000 IU; vit E 800; vit K 200; vit B1 200; vit B2 500; vit B6 50; vit B12 1200µg; vit C 2500; Ca-D pantothenate 600; niacin 4000; choline chloride 1000 methionine 3000; lysine 3000; manganese 12000; iron 2000; iodine 20; zinc 10000; coal 20; copper 400; santonium 1000; zinc bacitracin 2100. b) Laboratory of Feed Technology, Bogor Agricultural University (2015). c) Laboratory of Dairy Nutrition, Bogor Agricultural University (2015).

Table 2. Crude protein and ether-extract digestibility coefficients of the experimental broiler chickens fed diets supplemented with various doses of vitamin E and Zn

Variable	Zn (ppm)	Vitamin E (ppm)			Average
		0	125	250	
Crude protein digestibility (%)	0	75.67±4.92	73.23±6.78	74.01±7.10	74.44±5.39
	40	68.52±5.22	72.73±4.79	80.00±5.29	73.74±6.70
	80	81.00±0.70	77.47±1.29	78.79±6.84	79.29±4.01
	Average	75.07±6.51	74.23±4.53	77.60±6.23	
Ether extract digestibility (%)	0	86.18±1.60	86.45±6.01	88.19±4.96	86.83±3.44
	40	87.85±4.11	90.79±3.80	89.27±0.12	89.31±3.29
	80	88.53±3.21	90.95±2.41	89.51±1.68	89.50±2.36
	Average	87.52±2.92	89.60±4.05	89.06±2.33	

Carcass Traits

There was no significant interaction effect of Zn and vitamin E supplementations in the diet on carcass traits

(Table 3) and meat bone ratio of experimental broiler chickens (Table 4). There were significant interactions ($P<0.05$) between Zn and vitamin E supplementation on the final body weight of broiler. Supplementation

Table 3. Carcass traits of experimental broiler chickens fed diets supplemented with various doses of vitamin E and Zn

Variable	Zn (ppm)	Vitamin E (ppm)			Average
		0	125	250	
Final body weight (g)	0	1700.00±118.88 ^{ab}	1345.00±286.30 ^c	1730.00±124.90 ^{ab}	
	40	1735.00±99.83 ^{ab}	1645.00±50.00 ^{ab}	1605.00±41.23 ^b	
	80	1825.00±138.92 ^a	1565.00±59.72 ^b	1725.00±52.60 ^{ab}	
Body weight gain (g)	0	1434.18±78.05	1482.39±50.33	1494.91±79.74	1470.49±69.53
	40	1464.60±10.59	1542.43±145.05	1460.82±68.18	1513.83±92.41
	80	1566.20±43.85	1534.44±72.60	1511.01±63.32	1533.69±90.40
	Average	1566.20±98.49	1518.67±97.84	1511.01±63.32	
Carcass (%)	0	66.01±7.78	69.18±9.50	68.94±4.52	68.72±9.01
	40	66.07±3.81	71.27±9.91	66.89±6.09	68.08±3.08
	80	69.85±7.17	69.08±8.03	65.03±4.16	67.99±2.03
	Average	67.31±2.14	69.84±7.29	66.95±1.02	
Breast (%)	0	33.25±2.10	31.38±1.68	30.10±1.35	31.57±0.37
	40	33.57±2.18	33.12±2.38	31.53±2.88	32.74±0.36
	80	34.38±0.33	33.18±2.08	32.24±1.79	33.26±0.93
	Average	33.73±1.04	32.56±0.36	32.62±0.79	
Thigh (%)	0	30.27±1.06	33.07±2.86	31.45±1.78	31.60±0.91
	40	30.04±1.80	29.88±1.85	29.29±1.95	29.73±0.08
	80	30.72±1.88	30.90±1.42	30.89±0.70	30.84±0.59
	Average	30.34±0.46	31.28±0.74	30.54±0.68	
Wings (%)	0	12.13±1.93	12.99±1.67	12.30±1.01	12.47±0.48
	40	11.23±0.51	11.03±1.32	11.33±0.67	11.20±0.43
	80	11.24±0.88	11.90±0.39	12.03±0.61	11.72±0.25
	Average	11.53±0.74	11.97±0.67	11.89±0.21	
Back (%)	0	19.66±2.17	20.42±1.75	22.15±1.40	20.75±0.39
	40	19.64±1.23	21.27±0.96	19.96±1.24	20.29±0.16
	80	20.24±1.35	20.99±1.78	19.86±1.76	20.37±0.24
	Average	19.85±0.51	20.90±0.46	20.66±0.26	

Note: Means in the same column/raw with different superscripts differ significantly ($P<0.05$).

Table 4. Meat bone ratio (MBR) of breast at the age of 35 days in experimental broiler chickens fed diets supplemented with various doses of Zn and vitamin E

Variable	Zn (ppm)	Vitamin E (mg)			Average
		0	125	250	
Breast (g)	0	371.00±34.59	335.50±72.40	347.50±37.24	351.33±48.08
	40	386.25±26.29	387.00±50.51	336.50±15.84	369.92±30.88
	80	437.50±47.56	359.25±55.24	362.50±42.26	386.42±48.35
	Average	398.25±36.15	360.58±59.38	348.83±31.78	
Meat (g)	0	281.00±36.53	264.00±56.85	296.00±40.82	280.33±44.73
	40	314.25±27.29	301.25±24.38	265.00±19.88	293.50±23.85
	80	351.75±34.04	288.75±51.82	285.50±36.61	308.67±40.82
	Average	315.67±32.42	284.67±44.35	282.17±32.44	
Bone (g)	0	84.25± 7.63	69.25±15.41	51.00± 9.42	68.17±10.82
	40	70.75±18.37	83.75±25.36	68.25±17.86	74.25±20.53
	80	81.25±29.80	68.00±21.46	74.75±17.58	74.67±22.95
	Average	78.75±18.60	73.67±20.73	64.67±14.95	
Meat bone ratio (MBR)	0	3.34	3.81	5.80	
	40	4.44	3.60	3.88	
	80	4.33	4.25	3.82	

of Zn 80 ppm at vitamin E 0 ppm gave higher response compared to that of 125 ppm or 250 ppm. Interactions of supplementation of Zn 0 ppm and vitamin E 125 ppm yielded the lowest final body weight.

Mineral Availability

There was no significant interaction effect of Zn and vitamin E supplementations in the diet on the weights of tibia as well as the content of ash (Table 5), calcium, phosphorus and zinc of the tibia of experimental broiler chickens (Table 6). However, supplementation of zinc 40 ppm or 80 ppm significantly ($P<0.05$) increased calcium content of tibia of experimental broiler chickens. Supplementation of vitamin E 125 ppm or 250 ppm significantly increased ($P<0.05$) the calcium content of broiler chickens' tibia.

DISCUSSION

High ambient temperature can decrease feed digestibility values. Stress can cause gastrointestinal injuries (Burkholder *et al.*, 2008) and, studies of Liu *et al.* (2009) and Yu *et al.* (2010) found that heat stress caused damages to porcine intestinal epithelia, which

included the tips of the intestinal villi, inducing epithelial cell shedding, exposing the intestinal mucosa lamina propria, as well as the shortening of villus height and crypt depth. Heat stress would damage the barrier to digestive tract, lower blood flow to the digestive tract to 50% as in the proventriculus, gizzard, and pancreas, while the rate of blood flow in the upper duodenum and jejunum decreased to 70% during heat stress (Gu *et al.*, 2012). In addition, Hao *et al.* (2012) reported that after 10 h of continuous heat exposure under $36\pm1^{\circ}\text{C}$, the amylase, lipase, and trypsin activities in the intestine of broilers significantly decreased at the age of 21 d. Supplementations of Zn and vitamin E somehow seemed to reduce the negative effect of the heat stress.

The treatments did not affect the crude protein and ether-extract digestibility, carcass traits, meat bone ratio, the weight of tibia and content of ash, phosphorus, zinc of tibia. However, there was interaction of supplementations of Zn and vitamin E on final body weight and calcium content in the tibia, digestibility of crude protein and ether-extract on the broiler chickens. It could be due to adequacy of Zn or vitamin E in the diet for the experimental broiler chickens.

The statistical analysis indicated that there was no significant effects of supplementation of Zn and vitamin

Table 5. The weight and ash contents of tibia of experimental broiler chickens fed diets supplemented with various doses of vitamin E and Zn

Variable	Zn (ppm)	Vitamin E (ppm)			Average
		0	125	250	
Tibia weight (g)	0	4.27±0.35	3.93±0.99	3.83±0.32	4.01±0.37
	40	4.40±0.20	4.03±0.50	3.83±0.46	4.09±0.16
	80	3.93±0.49	4.00±0.17	3.60±0.17	3.84±0.18
	Average	4.20±0.15	3.99±0.40	3.60±0.14	
Ash content (%)	0	39.96±2.92	41.58±1.69	45.00±4.24	41.98±1.27
	40	40.84±0.51	41.75±2.61	42.33±0.60	41.64±1.19
	80	44.33±2.81	41.51±3.69	43.56±2.88	43.13±0.49
	Average	41.51±1.36	41.61±1.00	43.63±1.84	

Table 6. Calcium, phosphorus, and zinc contents of the tibia in the experimental broiler chickens fed diets supplemented with various doses of vitamin E and Zn

Variable	Zn	Vitamin E			Average
		0	125	250	
Calcium (%)	0	41.35± 4.12	49.66± 1.84	50.06± 0.69	47.01± 1.74 ^b
	40	48.89± 0.52	49.84± 3.73	52.48± 1.11	50.40± 1.71 ^a
	80	50.30± 1.54	53.78±10.16	51.83± 1.18	51.97± 5.08 ^a
	Average	46.85± 1.86 ^b	51.09± 1.69 ^a	51.46± 0.27 ^a	
Phosphorus (%)	0	12.54± 1.92	13.61± 0.27	13.12± 0.18	13.09± 0.98
	40	13.61± 0.30	13.31± 0.16	13.14± 0.21	13.35± 0.07
	80	13.14± 0.26	13.51± 0.31	13.36± 0.24	13.34± 0.04
	Average	13.10± 0.95	13.47± 0.08	13.21± 0.03	
Zinc (ppm)	0	387.56±74.84	420.27±33.12	418.28±24.80	395.50±30.66
	40	427.42±20.33	415.56± 2.25	375.61±48.17	409.53±16.25
	80	371.53±23.28	392.77±26.47	393.91±16.29	395.94±16.50
	Average	408.70±26.81	406.20±23.13	386.07± 5.21	

Note: Means in the same column/raw with different superscripts differ significantly ($P<0.05$).

E in combination on apparent ether-extract digestibility. However, the combination of supplementation of Zn and vitamin E tended to increase the values of apparent ether-extract digestibilities. In contrast, previous studies reported by Sahin and Krucuk (2003) stated that supplementation of Zn in quail diet, increased apparent nutrient digestibility. Recent study reported that the percentages of Zn absorption, both as inorganic and organic Zn sources in the ileum, were higher than in the duodenum and jejunum (Yu *et al.*, 2008; Yu *et al.*, 2010). Sahin & Kucuk (2001) reported that the digestibilities of nutrients (dry matter, organic matter, crude protein, and ether extract) increased with the increasing levels of dietary vitamin E. The increased nutrient digestibilities was probably due to the role of Zn in protecting pancreatic tissues from oxidative damage (Onderci *et al.*, 2003), so that the pancreas functioned optimally to produce the digestive enzymes that would increase the digestibility of nutrient. Onderci *et al.* (2003) reported that supplementation of chromium and zinc decreased the digestibilities of dry matter, crude protein, and ether extract in laying hens maintained in a low temperature environment.

Heat stress can decrease body weight gain as well as feed intake and feed conversion ratio in broiler chickens (Rozenboim *et al.*, 2007; Star *et al.*, 2008; Quinteiro-Filho *et al.*, 2010). Supplementation of Zn at a dose of 80 ppm in the diet without vitamin E supplementation significantly increased final weight of broiler chickens. Zaghari *et al.* (2015) reported that the increase in Zn concentration of the diet by supplementing the basal diet, (with original Zn concentration of 24 mg/kg) with additional Zn at a dose of 30 mg/kg significantly increased the final body weights of male broiler chickens. The male broiler chickens fed diets with zinc concentrations of 60.1 and 57.4 mg/kg showed the highest final body weight. Huang *et al.* (2007) suggest that the optimal dietary zinc level of chicks from hatch to 21 d of age is 84 mg/kg. In contrast, supplementation of combination of Zn and vitamin E in the diets did not affect the body weight gain of broiler chickens. Broiler chickens fed diet supplemented with Zn at a dose of 80 ppm without vitamin E supplementation had higher body weight gains. Many studies reported that supplementary zinc did not affect growth performance such as body weight gain, feed intake, and feed conversion ratio (Kakhki *et al.*, 2016; Liu *et al.*, 2011, Yang *et al.*, 2016). In a recent study, heat stress in broilers can lead to a decreased feed intake, reduced weight, and increased feed conversion ratio (Sohail *et al.*, 2012). Supplementation of Zn as an antioxidant has reduced the negative effects of heat stress.

Supplementation of Zn or vitamin E as well as their combinations did not significantly affect the carcass traits (the percentage weight of carcass, breast, thighs, wings, and backs). Similar results were also reported by Khaki *et al.* (2016) that the broiler chickens fed diet with combination of zinc and α -tocopheryl acetate (α -TOA) did not show any change in the yields of carcass parts. Rossi *et al.* (2007) also stated that carcass yields were not influenced by the increasing levels of dietary organic Zn

of the broiler chickens. The statistical analysis indicated that the supplementations of Zn and vitamin E in combination in the diets of broiler chickens did not affect breast meat, breast bone, and meat bone ratio. Zhang *et al.* (2012) reported that broilers raised in chronic high temperature had lower proportion of breast muscle and higher proportion of thigh muscle.

Supplementations of Zn and vitamin E in combination did not significantly affect the weight of the tibia and ash percentage of the tibia in broiler chickens at the age of 35 days. Similar results were reported by Sunder *et al.* (2008) who showed that supplementation of Zn at the level of 0 to 160 ppm did not affect the weight of the tibia and ash content of the tibia. Vakili *et al.* (2013) reported that the use of Zn and vitamin E did not give a significant effect on the ash content and weight of the tibia bone. According to Sahraei *et al.* (2012), the supplementation of Zn exceeded the recommended levels by as much as 40 to 70 ppm would not affect the levels of ash in the tibia, because the supplies of calcium and phosphorus as constituents of bone were already sufficient.

Heat stress significantly decreases the ash, Ca, and P contents of the tibia bone and leads to the decreased bone strength (Sahin *et al.*, 2006; Hosseini-Vashan *et al.*, 2016). Diets supplemented with a combination of Zn and vitamin E did not affect calcium content of the tibia. Supplementation of Zn alone from 40 to 80 ppm significantly increased calcium content of tibia bone. The most high calcium levels (51.97%) was found when the experimental broiler chickens were supplemented with 80 ppm of Zn. This result was in accordance with research conducted by Sunder *et al.* (2008) that zinc supplementation at a dose of 80 ppm produced a high calcium content up to 37.41%. Supplementation of vitamin E alone at the levels of 125 to 250 ppm significantly increased calcium content of tibia bone. Vitamin E supplementation of the diet at a dose of 125 ppm was more applicable in increasing the calcium content of tibia as compared to that of 250 ppm, because the supplementation at the two levels did not show significant differences. Lohakare *et al.* (2005) showed that calcium retention was higher when vitamin E was added at higher levels.

Supplementation of Zn and vitamin E in combination did not affect the phosphorus and zinc contents of the tibia, although, a recent study showed that dietary supplementation of zinc significantly affected phosphorus contents of the tibia (Sunder *et al.*, 2008; Vieira *et al.*, 2013). Supplementation of zinc at a dose of 40 ppm produced optimum levels of phosphorus by 17.59%. Sunder *et al.* (2008) suggested that bone zinc was a functional reserve of zinc of the body and it could easily be mobilized to any urgent needs by tissues. Supplementation of vitamin E did not affect the phosphorus and zinc content of the tibia. According to Mansoub *et al.* (2010), vitamin E and zinc are nutrients that are closely related to heat stress problems. Both of them are superoxide dismutase enzyme cofactors for superoxide anion break down into oxygen and hydrogen peroxide. This leads to the possibility that heat stress stimulate the mobilization of zinc, hence, provision of vitamin E in this study had no significant effect.

CONCLUSION

Supplementation of 80 ppm of Zn increased calcium content compared to control, but did not affect final weight and ether-extract digestibility. Supplementation of vitamin E at a dose of 125 ppm could increase calcium content of the tibia compared to control.

ACKNOWLEDGEMENT

The authors would like to thank Directorate General of Higher Education of Indonesia for supporting this research through Funding Research Program-BOPTN (Project ID No. 595/IT3.11/PT/2015).

REFERENCES

- AOAC. 2005. Official Methods of Analysis of Association of Official Analytical Chemists. 16th ed. Assoc. Off. Anal. Chem., Arlington.
- [BPS] Badan Pusat Statistik. 2015. Konsumsi rata-rata per kapita seminggu beberapa macam bahan makanan penting 2007-2014. <https://www.bps.go.id/linkTabelStatis/view/id/950>. [8 August 2016]
- Burkholder, K. M., K. L. Thompson, E. Einstein, T. J. Applegate & J. A. Patterson. 2008. Influence of stressors on normal intestinal microbiota, intestinal morphology and susceptibility to *Salmonella enteritidis* colonization in broilers. *Poult. Sci.* 87:1734-1741. <https://doi.org/10.3382/ps.2008-00107>
- Farrel, D. J. 1978. A nutritional evaluation of buckwheat (*Fagopyrum esculentum*). *Anim. Feed. Sci. Technol.* 3(2):95-108. [https://doi.org/10.1016/0377-8401\(78\)90038-X](https://doi.org/10.1016/0377-8401(78)90038-X)
- Gu, X. H., Y. Hao & X. L. Wang. 2012. Over-expression of heat shock protein and its relationship to intestine under acute heat stress in goat. *Int. J. Livest.* 3: 27-28.
- Habibian, M. S., Ghazi, M.M. Moeini & A. Abdolmohammadi. 2014. Effect of dietary selenium and vitamin E on immune response and biological blood parameters of broiler reared under thermoneutral or heat stress condition. *Int. J. Biomet.* 58(5): 741-752. <https://doi.org/10.1007/s00484-013-0654-y>
- Hao, Y. X. H. Gu & X. L. Wang. 2012. Overexpression of heat shock protein 70 and its relationship to intestine under acute heat stress in broilers: 1. Intestinal structure and digestive function. *Poult. Sci.* 91:781-789. <https://doi.org/10.3382/ps.2011-01627>
- Hashizawa, Y., M. Kubota, M. Kadowaki & S. Fujimura. 2013. Effect of dietary vitamin E on broiler meat qualities, color, water-holding capacity and shear force value under heat stress condition. *Anim. Sci. J.* 80: 732-736. <https://doi.org/10.1111/asj.12079>
- Hosseini-Vashan, S. J., A. Golian, & A. Yaghobfar. 2016. Growth, immune, antioxidant, and bone responses of heat stress-exposed broilers fed diets supplemented with tomato pomace. *Int. J. Biometeorol.* 60:1183-1192. <https://doi.org/10.1007/s00484-015-1112-9>
- Huang, Y. L., L. Lu, X. G. Luo & B. Liu. 2007. An optimal dietary zinc level of broiler chicks fed a corn-soybean meal diet. *Poult. Sci.* 86:2582-2589. <https://doi.org/10.3382/ps.2007-00088>
- Khakhi, R. A. M., R. Bakhshalinejad, A. Hassanabadi, & P. Ferket. 2016. Effect of dietary organic zinc and α -tocopheryl acetate supplements on growth performance, meat quality, tissue minerals, and α -tocopherol deposition in broiler chickens. *Poult. Sci.* 95:386. <https://doi.org/10.3382/ps/pew386>
- Lara, L. J & M. H. Rostagno. 2013. Impact of heat stress on poultry production. *Animals.* 3:356-369. <https://doi.org/10.3390/ani3020356>
- Leeson, S & J. D. Summers. 2008. Commercial Poultry Nutrition. 3rd ed. Department of Animal and Poultry Science, University of Guelph. University Books., Guelph.
- Liu, F., J. Yin, M. Du, P. Yan, J. Xu, X. Zhu, & J. Yu. 2009. Heat-stress-induced damage to porcine small intestinal epithelium associated with downregulation of epithelial growth factor signaling. *J. Anim. Sci.* 87:1941-1949. <https://doi.org/10.2527/jas.2008-1624>
- Liu, Z., L. Lu, S. Li, Z. Zhang, L. Xi, K. Zhang, & X. Luo. 2011. Effects of supplemental zinc source and level on growth performance, carcass traits and meat quality of broilers. *Poult. Sci.* 90:1782-1790. <https://doi.org/10.3382/ps.2010-01215>
- Liu, Z. H., L. Lu, R. L. Wang, L. Lei, S. F. Li, L. Y. Zhang, & X. G. Luo. 2015. Effects of supplemental zinc source and level on antioxidant ability and fat metabolism-related enzymes of broilers. *Poult. Sci.* 94: 2686-2694. <https://doi.org/10.3382/ps/pev251>
- Lohakare, J. D., Y. J. Choi, J. K. Kim, J. S. Yong, Y. H. Shim, T. W. Hahn, & B. J. Chae. 2005. Effect of dietary combinations of vitamin A, E, methionine on growth performance, meat quality and immunity in commercial broilers. *J. Anim. Sci.* 18(4): 516-523.
- Mansoub, N. H., S. C. Azar, A. A. Tehrani, A. Lotfi, & M. K. Manesh. 2010. Influence of dietary vitamin E and zinc on performance, oxidative stability and some blood measures of broiler chickens reared under heat stress. *J. Agrobiol.* 27(2): 103-110.
- Onderci, M., N. Sahin, K. Sahin, & N. Kilic. 2003. The antioxidant properties of chromium and zinc: in vivo effects on digestibility, lipid peroxidation, antioxidant vitamins and some minerals under a low ambient temperature. *Biol. Trace. Elem. Res.* 92: 139-150. <https://doi.org/10.1385/BTER.92.2:139>
- Quinteiro-Filho, W. M., A. Riberio, V. Ferraz-de-Paula, M. L. Pinheiro, M. Sakai, L. R. M. Sa, A. J. P. Ferreira, & J. Palermo-Neto. 2010. Heat stress impairs performance parameters, induces intestinal injury, and decreases macrophage activity in broiler chickens. *Poult. Sci.* 89:1905-1914. <https://doi.org/10.3382/ps.2010-00812>
- Rossi, P., F. Rutz, M. A. Anciuti, J. L. Rech, & N. H. F. Zauk. 2007. Influence of graded levels of organic zinc on growth performance and carcass traits of broilers. *J. Appl. Poult. Res.* 16: 219-225. <https://doi.org/10.1093/japr/16.2.219>
- Rozenboim, I., E. Tajo, O. Gal-Garber, J. A. Proudman, & Z. Uni. 2007. The effect of heat stress on ovarian function of laying hens. *Poult. Sci.* 86:1760-1765. <https://doi.org/10.1093/ps/86.8.1760>
- Rusli, R. K., K. G. Wiryawan, T. Toharmat, Jakaria, & R. Mutia. 2015. Effect of mangosteen pericarp meal and vitamin E supplements on the performance, blood profiles, antioxidant enzyme and HSP 70 gene expression of laying hens in tropical environment. *Int. J. Poult. Sci.* 14(10):570-576. <https://doi.org/10.3923/ijps.2015.570.576>
- Sahin, K & O. Kucuk. 2001. Effects of vitamin E and selenium on performance, digestibility of nutrients, and carcass characteristics of Japanese quails reared under heat stress (34 °C). *J. Anim. Physiol. a. Anim. Nutr.* 85: 342-348. <https://doi.org/10.1046/j.1439-0396.2001.00340.x>
- Sahin, K & O. Kucuk. 2003. Heat stress and dietary vitamin supplementation of poultry diets. *Nutr. Abstr. Rev. Ser. B. Livest. Feeds. Feeding.* 73: 41-50.
- Sahin, K., M. Onderci, N. Sahin, T. A. Balci, M. F. Gursu, V. Juturu, & O. Kucuk. 2006. Dietary arginine silicate inositol complex improves bone mineralization in quail. *Poult. Sci.* 85:486-492. <https://doi.org/10.1093/ps/85.3.486>

- Sahin, N, C. Orhan, M. Tuzcu, K. Sahin, & O. Kucuk. 2008. The effect of tomato powder supplementation on performance and lipid peroxidation in quail. *Poult Sci.* 87:267-283. <https://doi.org/10.3382/ps.2007-00207>
- Sahrei, M, H. Janmmohamadi, A. Taghizadeh, & S. Cheraghi. 2012. Effect of different zinc sources on tibia bone morphology and ash content of broiler chickens. *Advan. Biol. Res.* 6(3): 128-132.
- Salim, H. M, C. Jo, & B. Lee . D. 2008. Zinc in broiler feeding and nutrition. *Avian. Bio. Res.* 1: 5-18. <https://doi.org/10.3184/175815508X334578>
- Salim, H, H. Lee, C. Jo, S. Lee, & B. Lee. 2012. Effect of dietary zinc proteinate supplementation on growth performance and skin and meat quality of male and female broiler chicks. *Br. Poult. Sci.* 53:116-124. <https://doi.org/10.1080/00071668.2012.658757>
- Selim, N. A, S. F. Youssef, A. F. Abdel-Salam, & S. A. Nada. 2013. Evaluation of some natural antioxidant sources in broiler diets: 1-effect on growth, physiological, microbiological and immunological performance of broiler chicks. *Int. J. Poult. Sci.* 12:561-571. <https://doi.org/10.3923/ijps.2013.561.571>
- Sohail, M. U, M. E. Hume, J. A. Byrd, D. J. Nisbet, A. Ijaz, A. Sohail, M. Z. Shabbir, & H. Rehman. 2012. Effect of supplementation of prebiotic mannan-oligosaccharides and probiotic mixture on growth performance of broilers subjected to cronic heat stress. *Poult. Sci.* 91:2235-2240. <https://doi.org/10.3382/ps.2012-02182>
- Star, L. B. Kemp. I. Van den Anker, & H. K. Parmentier. 2008. Effect of single or combined climatic and hygienic stress in four layer lines: 1. Performance. *Poult. Sci.* 87:1022-1030. <https://doi.org/10.3382/ps.2007-00142>
- Steel, R. G. D & J. H. Torrie. 1995. Prinsip dan Prosedur Statistika. Suatu pendekatan Biometrik. Terjemahan. 5th ed. Gramedia Pustaka Utama, Jakarta.
- Sudarman, A, Sumiati, & S. H. Solikhah. 2011. Performance and meat cholesterol content of broiler chickens fed Pluchea indica L. leaf meal reared under stress condition. *Med. Pet.* 34:64-68. <https://doi.org/10.5398/medpet.2011.34.1.64>
- Sunder, G. S, A. K. Panda, N. C. S. Gopinath, S. V. Rama Rao, M. V. L. N. Raju, M. R. Reddy, & K. Ch. Vijay. 2008. Effect of higher levels of zinc supplementation on performance, mineral availability and immune competence in broiler chickens. *J. Appl. Poult. Res.* 17: 79-85. <https://doi.org/10.3382/japr.2007-00029>
- Taussky, H. H & E. E. Shor. 1953. A microcolometric method for determination of inorganic phosphorus. *J. Biol. Chem.* 202(2): 85-675.
- Troine, W, S. Kinal, & B. Lubojemska. 2007. Effect of various forms of zinc applied in concentrate mixtures for broiler chickens on its bioavailability as well as meat composition and quality. *Poult. J. Food. Nutr. Sci.* 57: 577-581.
- Vakili, R, A. S. Rashidi, & S. Sobjanirad. 2010. Effects of dietary fat, vitamin E and zinc supplementation on tibia breaking strenght in female broilers under heat stress. *Afr. J. Agric. Res.* 5(23): 3151-3156.
- Vieira, M. M, A. M. L. Riberio, A. M. Kessler, M. A. Kunrath, & V. S. Ledur. 2013. Different sources of dietary zinc for broiler submitted to immunological, nutritional and environmental challenge. *J. Appl. Poult. Res.* 22: 855-861. <https://doi.org/10.3382/japr.2013-00753>
- Yang, W. L, Y. P. Chen, Y. F. Cheng, X. H. Li, R. Q. Zhang, C. Wen, & Y. M. Zhou. 2016. An evaluation of zinc bearing palygorskite inclusion on the growth performance, mineral content, meat quality and atioxidant status of broilers. *Poult. Sci.* 95:878-885. <https://doi.org/10.3382/ps/pev445>
- Yu, J, P. Yin, F. H. Liu, G. L. Cheng, K. J. Guo, A. Lu, X. Y. Zhu, W. L. Luan, & J. Q. Xu. 2010. Effect of heat stress on the porcine small intestine: A morphological and gene expression study. *Comp. Biochem. Physiol. A Mol. Intergr. Physiol.* 156:119-128. <https://doi.org/10.1016/j.cbpa.2010.01.008>
- Yu, Y, L. Lu, X. G. Luo, & B. Liu. 2008. Kinetics of zinc absorption by in situ ligated intestinal loops of broilers involved in zinc transportes. *Poult. Sci.* 87:1146-1155.
- Yu, Y, L. Lu, R. L. Wang, L. Xi, X. G. Luo, & B. Liu. 2010. Effect of zinc source and phytate on zinc absoption by in situ ligated intestinal loops of broilers. *Poult. Sci.* 89:2157-2165. <https://doi.org/10.3382/ps.2009-00486>
- Zaghari, M, M. Avazkhanllo, & M. Ganjkanlou. 2015. Reevaluation of male broilerzinc requirement by dose-response trial using practical diet with added exogenous phytase. *J. Agr. Sci. Tech.* 17: 333-343.
- Zhang, Z. Y, G. Q. Jia, J. J. Zuo, Y. Zhang, J. Lei, L. Ren, & D. Y. Feng. 2012. Effect of constant and cyclic heat stess on muscle metabolism and meat quality of broiler breast fillet and thigh meat. *Poult. Sci.* 91:2931-2937. <https://doi.org/10.3382/ps.2012-02255>